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(54) Title: IMPLANTABLE DEVICE CONTAINING INHIBITOR OF MACROPHAGE MIGRATION INHIBITORY FACTOR

(57) Abstract: The present invention provides an implantable device, particularly a stent, comprising: (i) a reservoir containing at least one MIF inhibitor; and (ii) means to release or elute the inhibitor from the reservoir. Also disclosed are methods of treatment of diseases associated with MIF cytokine activity using the implantable device.

Implantable device containing inhibitor of macrophage migration inhibitory factor

FIELD OF THE INVENTION

The present invention is generally concerned with the field of implantable medical devices , in particular it is concerned with the use of implantable medical devices in the treatment of diseases that can be treated by the inhibition of the cytokine or biological activity of macrophage migration inhibitory factor (MIF).

BACKGROUND OF THE INVENTION

Macrophage migration inhibitory factor (MIF) is the first identified soluble lymphokine. MIF was first described as a soluble factor with the ability to modify the migration of macrophages (1). The molecule responsible for the biological actions ascribed to MIF was identified and cloned in 1989 (2). Initially found to activate macrophages at inflammatory sites, it has been shown to possess pluripotential actions in the immune system. MIF has been shown to be expressed in human diseases which are characterised by inflammation, injury, ischaemia, proliferation or malignancy. MIF also has a unique relationship with glucocorticoids in that MIF is induced by glucocorticoids but acts through overriding their anti-inflammatory effects.

Recent studies have indicated that antagonism of MIF may be useful in the treatment of sepsis, certain types of cancers and proliferative diseases, and inflammatory diseases. Monoclonal antibody antagonism of MIF has been shown to have activity in adjuvant- or collagen-induced arthritis animal models, and other models of inflammatory and immune diseases, including vascular disease and ischaemia, have also been shown to be influenced by MIF.

There is a broad spectrum of effects of MIF within the immune system, including actions in the regulation of innate and adaptive immunity. The absence of MIF or its blockade using antibodies is associated with suppression of the host animal's response to bacterial endotoxin and exotoxins (3). In view of the effects of MIF on the immune system, systemic therapeutic antagonism of the biological or cytokine activity of MIF can also be hypothesised to have systemic inhibitory effects on the immune system of the host. This may be desirable in the case of systemic immune and inflammatory diseases. This may be undesirable, in contrast, in the case of diseases or conditions where the pathological activity of MIF is restricted to a local region such as a local lesion, tissue or organ. In addition, systemically administered drugs may not be able to reach sufficient concentrations in local or regional sites to achieve

the desired therapeutic effect (4). Moreover, in line with the generally accepted principle of using the minimum effective amount of any therapeutic agent, delivery of a MIF antagonist therapy to a local region would permit the use of lower total doses of MIF antagonist to achieve a desired effect compared to systemic administration. There exists a need, therefore, for therapeutic antagonism of MIF which can be delivered locally.

One means by which the local inhibition of MIF can be effected is the use of implanted devices that would elute an inhibitor of the cytokine or biological activity of MIF. Implanted devices which elute a pharmaceutical active are known in the art and are used to distribute the active either systemically, as is the case with implantable systemic drug delivery devices such as Implanon ®, or locally. There are a variety of possible forms of such devices including: an implanted crystalline form of the active which releases the active by slow dissolution (for example crystalline corticosteroid preparations such as Depo-Medrol®); polymeric pellets impregnated with the pharmaceutical active; implantable pumps which distribute the active from a reservoir and vascular stents impregnated with the active (for example, Sirolimus-eluting coronary stents (4)).

To date, there have been no examples of implanted devices that elute an inhibitor of the cytokine or biological activity of MIF. Although antibody antagonism of MIF is one potential way to provide therapeutic treatments, such biological molecules can be expensive to prepare on a commercial basis, are limited in the way they are administered (generally by injection), and do not readily lend themselves to formulations for administration by other means. Because of the specific physicochemical requirements for therapeutic actives delivered via implantable devices or similar means, these molecules may not be appropriate for local administration into a lesion via an implanted device.

Small molecule inhibitors may overcome one or more such difficulties connected with the use of biological therapeutic treatments. They can be formulated for delivery via devices such as implants or implanted devices.

It has been suggested that atherosclerotic vascular disease is connected with the presence of MIF (5). Atherosclerotic vascular disease is associated with features of inflammation within atheromatous lesions in vessel walls, and is a common cause of organ or tissue ischaemia and infarction. It is a disease predominantly associated with localised, as opposed to systemic, activation of the immune system.

Atherosclerotic vascular disease, and vascular diseases generally, can result in the narrowing or blocking of affected vessels. A common existing treatment is the insertion of vascular stents to maintain vessel diameter after dilation of the vessel by balloon angioplasty.

Although stents can be used to restore function to a diseased vessel, they do not treat the underlying causes of a disease such as atherosclerotic vascular disease. For example, atherosclerotic vascular disease in the vessel at or distal to the stented area may limit blood flow in that vessel and result in ischaemia and/or infarction of the vascular territory supplied by the stented vessel.

Furthermore, over the course of time stents have a tendency to fail. Stent restenosis is a common reason for failure of stents, and is the result of the process of neo-intimal hyperplasia (4). Neo-intimal hyperplasia is known to be dependent on vascular cell proliferation, the local activation of leukocyte recruitment, and cell activation via pathways known as mitogen-activated protein (MAP) kinases (4). These processes are also observed in angiogenesis.

In particular, studies suggest that stent restenosis is largely a result of vascular smooth muscle cell (VSMC) proliferation which is exaggerated after stent deployment due to the high-pressure technique of stent deployment.

It is therefore advantageous to use stents that are associated with positive impact on residual vascular disease in the treated vessel, such as atherosclerotic disease in the treated vessel, as well as a reduction in the progress of neo-intimal hyperplasia.

MIF is known to be expressed in atheroma lesions, and is able to induce endothelial and macrophage cell activation in ways likely to be important in the development or persistence of atheroma (5). Limitation of the biological effects of MIF on cells which participate in the development or persistence of atheroma can therefore be hypothesised to be of benefit in the limitation of atheroma and atherosclerotic vascular disease. MIF is also known to contribute to acute atherosclerotic plaque rupture, such as is seen in critical ischaemic events (6).

As the inventors have shown, MIF antagonism *in vivo* is indeed able to limit atheroma, consistent with a potential therapeutic effect on atherosclerotic vascular disease such as is seen in coronary and other vessels treated with stents.

MIF is also known to induce all the phenomena associated with the neo-intimal hyperplasia seen in stent restenosis, including vascular cell proliferation (7, 8), leukocyte recruitment, and MAP kinase activation (9). Limitation of the inflammatory and proliferative effects of

MIF can therefore be hypothesised to be of benefit in conditions characterised by unwanted vascular proliferation or inflammation, such as in atheroma, atherosclerotic plaque rupture, or vascular stent restenosis.

SUMMARY OF THE INVENTION

The present inventors have found that that compounds of Formula I and II may inhibit the cytokine or biological activity of MIF. They have further found that compounds that act as MIF inhibitors are effective in the treatment of atheroma and act to inhibit vascular smooth muscle cell proliferation. Such compounds may be usefully administered locally via implants or implanted devices.

Accordingly, in a first aspect, the present invention provides an implantable device comprising:

(i) a reservoir containing at least one MIF inhibitor; and

(ii) means to release or elute the inhibitor from the reservoir

In a second aspect, the present invention provides a method for inhibiting the cytokine or biological activity of MIF in a subject comprising the step of implanting a device according to the first aspect in the subject.

Preferably, the method is for inhibiting the cytokine or biological activity of MIF in a local region of the subject and the device is implanted within or proximate to the local region of the subject.

In a third aspect, the present invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine activity is implicated comprising the step of implanting a device according to the first aspect in a subject in need thereof.

Preferably, the disease or condition is confined to a local region of the subject and the device is implanted within or proximate to the local region.

In a fourth aspect the present invention provides an angioplastic stent operably coated with a prophylactically effective dose of a composition comprising at least one MIF inhibitor.

In a fifth aspect, the present invention provides a method for inhibiting the onset of restenosis in a subject undergoing angioplasty, which comprises topically administering a stent according to the fourth aspect to the subject at around the time of the angioplasty.

In a sixth aspect, there is provided a method of reducing the severity of stent restenosis in the vicinity of a stent comprising the use of a stent according to the fourth aspect.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows MIF mRNA expression as measured by RT-PCR in samples obtained from the aorta of Apo-E -/- mice, as described. Lane 1: size markers. Lane 2: 22 amplification cycle RT-PCR product from the aorta of a 12-week high fat diet-fed Apo-E mouse. Lane 3: 22 amplification cycle PCR (ie no reverse transcription) product from the aorta of a 12-week high fat diet-fed Apo-E mouse. Lane 4: 25 amplification cycle RT-PCR product from the aorta of a 12-week high fat diet-fed Apo-E -/- mouse. Lane 5: 25 amplification cycle PCR (ie no reverse transcription) product from the aorta of a 12-week high fat diet-fed Apo-E mouse. MIF mRNA expression was detectable in aortas of high fat diet-fed Apo-E -/- mice.

Figure 2 shows the results of immunostaining for the expression of MIF protein in sections of aorta from Apo-E -/- mice fed a high fat diet.

Figure 3 shows the effect of a compound of formula III 15 mg/kg/day, administered in once-daily doses by oral gavage, on atheroma lesion area as measured by Oil Red-O staining. III treatment was associated with a significant reduction in lesion area (*$p = 0.002$).

Figure 4 shows the effect of a compound of formula III 15 mg/kg/day, administered in once-daily doses by oral gavage, on atheroma lesion severity as measured by macrophage infiltration, as analysed by anti-CD68 mAb immunohistochemical staining. CD68 is a marker specific for macrophages. III treatment was associated with a significant reduction in lesion macrophage numbers ($p=0.022$).

Figure 5 shows an analysis of plasma cholesterol in ApoE -/- mice prior to being fed high fat diet (T0) or after 8 weeks of high fat diet plus either vehicle or a compound of formula III 15 mg/kg/day administered by once daily oral gavage. Increased plasma cholesterol was observed in response to high fat diet, with no significant difference between vehicle treated mice and mice treated with a compound of formula III.

Figure 6 shows the effects of serum on the proliferation of murine vascular smooth muscle cells (VSMC) measured using CellTiterGlo and expressed as relative luminescence units (RLU). Treatment of vascular smooth muscle cells with 10% FBS induced a significant increase in total cellular ATP pool levels as determined by the increase in RLU value relative to 0.5% FBS ($P<0.001$).

Figure 7 shows the effects of recombinant human MIF on the proliferation of murine vascular smooth muscle cells (VSMC) measured using CellTiterGlo and expressed as relative luminescence units (RLU). MIF induced a significant increase in VSMC proliferation (*P<0.02, *P<0.01) relative to untreated cells.

Figure 8 shows the concentration of MIF in conditioned medium from cultures of murine vascular smooth muscle cells. MIF is produced by murine vascular smooth muscle cells with significantly greater MIF production by cells cultured in 10% FBS.

Figure 9 shows the effects of pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate on the proliferation of murine vascular smooth muscle cells (VSMC) cultured in 10% FBS, measured using CellTiterGlo and expressed as relative luminescence units (RLU). Compared to control-treated cells, pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate induced a significant, dose-dependent decrease in VSMC proliferation (*P<0.05). This effect approached the effect of culturing cells in 0.5% FBS, as shown.

Figure 10 shows the effects of 2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide on the proliferation of murine vascular smooth muscle cells (VSMC) cultured in 10% FBS, measured using CellTiterGlo and expressed as relative luminescence units (RLU). Compared to control-treated cells, 2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide induced a significant, dose-dependent decrease in VSMC proliferation (*P<0.05). This effect approached the effect of culturing cells in 0.5% FBS, as shown.

Figure 11 shows the release of pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate from a phosphorylcholine coated coronary stent, into phosphate buffered saline solution (3.0 ml) at room temperature. Absorbance measurements were made at 287 nm and converted to concentrations with a standard curve of absorbance versus concentration using phosphate buffered saline as a reference. The time required to release 95% of the material from the stent was 94 minutes.

Figure 12 shows the release of 2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide from a phosphorylcholine coated coronary stent, into phosphate buffered saline solution (2.8 ml) at room temperature. Absorbance measurements were made at 266 nm and converted to concentrations with a standard curve of absorbance versus concentration using phosphate buffered saline as a reference. The time required to release 95% of the material from the stent was 91 minutes.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the present invention provides an implantable device comprising:

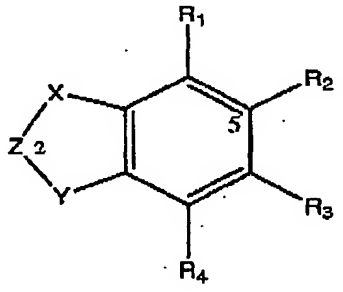
(i) a reservoir containing at least one MIF inhibitor; and

(ii) means to release or elute the inhibitor from the reservoir.

The term "reservoir" includes any means by which the MIF inhibitor is incorporated into the implantable device so that it can be released or eluted. It includes, but is not limited to, implanted crystalline forms of the MIF inhibitor, impregnation of the MIF inhibitor into polymeric pellets, reservoirs contained in implantable pumps, and coating or impregnating the MIF inhibitor onto or into the surface of the device.

The "means to release or elute the inhibitor" includes, but is not limited to, mechanisms such as implantable pumps and biodegradable polymers. Also contemplated is simple exposure of the MIF inhibitor to the physiological environment such as occurs with implanted crystalline forms of the active where the active is released by slow dissolution. The person skilled in the art would be aware of many possible means by which the MIF inhibitor may be released or eluted from the device.

As used herein the term "MIF inhibitor" means a compound of the general formula (I) or (II), or a pharmaceutically acceptable salt or prodrug thereof:



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wherein

X is selected from -O-, -S-, $-C(R_5)(R_5)-$ or $-N(R_6)-$ and preferably comprises a hydrogen bond donor or acceptor;

Y is selected from -N(R_7)-, -O-, -S- or -C(R_7)$_2$-;

Z is selected from -C(O)-, -C(S)-, -C(=NR_6)-, -S(O)- or -S(O)$_2$-;

R_1 is selected from hydrogen, C_{1-3}alkyl, ($CR_5R_{5'}$)$_n$OR_7, ($CR_5R_{5'}$)$_n$SR_7, ($CR_5R_{5'}$)$_n$N(R_6)$_2$ and ($CR_5R_{5'}$)$_n$halo;

R_2 is selected from C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, ($CR_{12}R_{12'}$)$_m$C(O)R_8, ($CR_{12}R_{12'}$)$_m$C(S)R_8, ($CR_{12}R_{12'}$)$_m$S(O)R_8, ($CR_{12}R_{12'}$)$_m$S(O)$_2R_8$, ($CR_{12}R_{12'}$)$_m$OR_9, ($CR_{12}R_{12'}$)$_m$SR_9, ($CR_{12}R_{12'}$)$_m$N$R_{10}R_{11}$, ($CR_{12}R_{12'}$)$_m$C(=NR_{24})R_{22} and ($CR_{12}R_{12'}$)$_m$$R_{13}$;

R_3 is selected from hydrogen, C_1-C_6alkyl, ($CR_{16}R_{16'}$)$_p$N$R_{14}R_{15}$, ($CR_{16}R_{16'}$)$_p$OR_{17}, ($CR_{16}R_{16'}$)$_p$SR_{17}, ($CR_{16}R_{16'}$)$_p$halo, ($CR_{16}R_{16'}$)$_p$NO_2, ($CR_{16}R_{16'}$)$_n$C(O)R_{28}, ($CR_{16}R_{16'}$)$_n$C(=NR_{24})R_{22}, ($CR_{16}R_{16'}$)$_n$S(O)R_{17}, ($CR_{16}R_{16'}$)$_n$S(O)$_2R_{17}$, ($CR_{16}R_{16'}$)$_n$S(O)$_3R_{17}$ and ($CR_{16}R_{16'}$)$_p$C(R_{18})$_3$;

R_4 is selected from hydrogen, halogen C_1-C_3alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl and ($CR_{12}R_{12'}$)$_n$C(R_{18})$_3$;

Each R_5 and $R_{5'}$ is independently selected from hydrogen, C_1-C_3alkyl, halo, OR_7, SR_7 and N(R_6)$_2$;

Each R_6 is independently selected from hydrogen, C_1-C_3alkyl and OR_7;

Each R_7 is independently selected from hydrogen and C_1-C_3alkyl;

R_8 is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, OR_{19}, SR_{19}, N(R_{20})$_2$, [NH-CH(R_{21})-C(O)]$_q$-OR_{29}, [sugar]$_q$ and ($CR_{12}R_{12'}$)$_t$$R_{13}$;

R_9 is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, ($CR_{12}R_{12'}$)$_t$$R_{13}$, C(O)$R_{23}$, C$O_2R_{23}$, C(S)$R_{23}$, C(S)O$R_{23}$, S(O)$R_{23}$, S(O)$_2R_{23}$, [C(O)CH($R_{21}$)NH]$_q$-$R_{23}$ and [sugar]$_q$;

R_{10} and R_{11} are independently selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, ($CR_{12}R_{12'}$)$_m$$R_{13}$, C(O)$R_{23}$, C(S)$R_{23}$, S(O)$R_{23}$, S(O)$_2R_{23}$, [C(O)CH($R_{21}$)NH]$_q$-$R_{23}$, -[sugar]$_q$ and NHC(=NR_{25})-NH_2;

Each R_{12} and $R_{12'}$ is independently selected from hydrogen, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, OR_{24}, SR_{24}, halo, N(R_{24})$_2$, CO_2R_{24}, CN, NO_2, aryl or heterocyclyl;

R_{13} is selected from OR_{25}, SR_{25}, halo, N(R_{25})$_2$, C(O)R_{31}, CN, C(R_{18})$_3$, aryl or heterocyclyl;

R_{14} and R_{15} are independently selected from hydrogen, C_1-C_3alkyl, OR_{17}, ($CR_{16}R_{16'}$)$_p$C(R_{18})$_3$;

Each R_{16} and $R_{16'}$ is independently selected from hydrogen, C_1-C_3alkyl, halo, OR_{17}, SR_{17} and $N(R_{17})_2$;

Each R_{17} is independently selected from hydrogen and C_1-C_3alkyl;

Each R_{18} is independently selected from hydrogen and halo;

R_{19} and each R_{20} are independently selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, $(CR_{26}R_{26'})_tR_{27}$;

R_{21} is the characterising group of an amino acid;

R_{22} is selected from C_1-C_6alkyl, NH_2, $NH(C_{1-6}alkyl)$, $N(C_{1-6}alkyl)_2$, OR_{29} or SR_{29};

R_{23} is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, aryl $(CR_{26}R_{26'})_tR_{27}$;

Each R_{24} is independently selected from hydrogen and C_1-C_6alkyl;

Each R_{25} is independently selected from hydrogen, C_1-C_6alkyl, C_{1-3}alkoxyC_{1-6}alkyl, aryl and heterocyclyl;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, OR_{29}, SR_{29}, halo, $N(R_{29})_2$, CO_2R_{29}, CN, NO_2, aryl and heterocyclyl;

R_{27} is selected from hydrogen, OR_{30}, SR_{30}, halo, $N(R_{30})_2$, CO_2R_{30}, aryl and heterocyclyl;

R_{28} is selected from hydrogen, C_{1-6}alkyl, OR_{29}, SR_{29} or $N(R_{29})_2$;

Each R_{29} is independently selected from hydrogen and C_1-C_3alkyl;

Each R_{30} is independently selected from hydrogen, C_1-C_3alkyl, aryl and heterocyclyl;

R_{31} is selected from C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

n is 0 or an integer from 1 to 3;

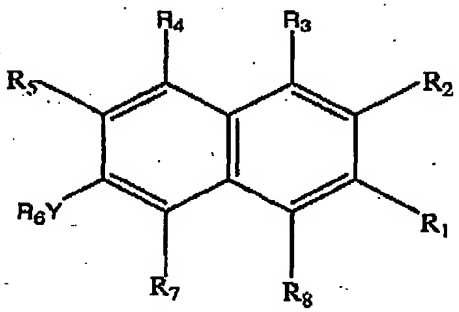
m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

q is an integer from 1 to 5;

t is an integer from 1 to 10;

wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted;



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wherein

Y is O, NR_9 or $S(O)_q$,

R_1 is selected from hydrogen, C_{1-6}alkyl, $-(CR_{10}R_{10'})_n$halo, $-(CR_{10}R_{10'})_nOR_{11}$, $-(CR_{10}R_{10'})_n-SR_{11}$, $-(CR_{10}R_{10'})_n-N(R_{12})_2$, $-(CR_{10}R_{10'})_nS(O)R_{11}$, $-(CR_{10}R_{10'})_nS(O)_2R_{11}$, $-(CR_{10}R_{10'})_n-S(O)_3R_{11}$, $-(CR_{10}R_{10'})_nC(O)R_{13}$, $-(CR_{10}R_{10'})_n-C(=NR_{14})R_{15}$ or $-(CR_{10}R_{10'})_nR_{16}$;

R_2 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $-(CR_{10}R_{10'})_mOR_{17}$, $-(CR_{10}R_{10'})_mSR_{17}$, $-(CR_{10}R_{10'})_mNR_{18}R_{19}$, $-(CR_{10}R_{10'})_mS(O)R_{20}$, $-(CR_{10}R_{10'})_mS(O)_2R_{20}$, $-(CR_{10}R_{10'})_mC(O)R_{20}$, $-(CR_{10}R_{10'})_mC(S)R_{20}$, $-(CR_{10}R_{10'})_mC(=NR_{11})R_{15}$ or $-(CR_{10}R_{10'})_mR_{16}$;

R_3, R_4 and R_5 are independently selected from hydrogen, C_{1-3}alkyl, $-(CR_{10}R_{10'})_nN(R_{14})_2$, $-(CR_{10}R_{10'})_nOR_{14}$, $-(CR_{10}R_{10'})_nSR_{14}$ or $-(CR_{10}R_{10'})_n$halo;

R_6 is selected from hydrogen, C_{1-6}alkyl, $-C(O)C_{1-6}$alkyl, $-C(O)N(R_9)_2$-, $-C(S)N(R_9)_2$- or $-(CR_{10}R_{10'})_nR_{21}$, or R_6Y and R_5 together may form $-X-(CH_2)_t-Z-$, where X and Z may be independently selected from O, S or NR_{14};

R_7 and R_8 are independently selected from hydrogen, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl or $-(CR_{10}R_{10'})_nR_{22}$;

Each R_9 is independently selected from hydrogen or C_{1-6}alkyl;

Each R_{10} and $R_{10'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl,

halogen, OR_{11}, SR_{11}, C_{1-3}alkoxy, CO_2R_{14}, $N(R_{14})_2$, CN, NO_2, aryl or heterocyclyl;

R_{11} is hydrogen or C_{1-6}alkyl;

Each R_{12} is independently selected from hydrogen, C_{1-6}alkyl, $C(=NR_{14})R_{15}$, $NH\text{-}C(=NR_{14})R_{15}$, $C(O)R_{14}$ or $C(S)R_{14}$;

R_{13} is hydrogen, C_{1-6}alkyl, OR_{14}, SR_{14} or $N(R_{14})_2$;

Each R_{14} is independently selected from hydrogen or C_{1-3}alkyl;

R_{15} is C_{1-6}alkyl, NH_2, $NH(C_{1-3}$alkyl) or $N(C_{1-3}$alkyl$)_2$, OR_{23} or SR_{23};

R_{16} is hydroxy, C_{1-3}alkoxy, SH, SC_{1-3}alkyl, halo, $C(O)R_{31}$, $C(R_{24})_3$, CN, aryl or heterocyclyl;

R_{17} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, CO_2R_{25}, $C(S)R_{25}$, $C(S)OR_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r\text{-}R_{23}$ or $[sugar]_r$;

R_{18} and R_{19} are independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, $C(S)R_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r\text{-}R_{23}$, $[sugar]_r$, $C(=NR_{23})NH_2$ or $NH\text{-}C(=NR_{23})NH_2$;

R_{20} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OR_{28}, SR_{28}, $N(R_{28})_2$, $[NH\text{-}CHR_{29}C(O)]_t\text{-}OR_{23}$, $[sugar]_r$, or $(CR_{26}R_{26'})_sR_{27}$;

R_{21} is OR_{28}, SR_{28}, halo or $N(R_{25})_2$;

R_{22} is halo, CO_2H, SO_3H, NO_2, NH_2, CO_2C_{1-3}alkyl, SO_3C_{1-3}alkyl or $C(R_{24})_3$;

R_{23} is hydrogen or C_{1-3}alkyl;

Each R_{24} is independently selected from hydrogen, Cl or F;

Each R_{25} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, aryl or $(CR_{26}R_{26'})_sR_{27}$;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, hydroxy, C_{1-3}alkoxy, SH, C_{1-3}alkylthio, CO_2H, CO_2C_{1-3}alkyl, NH_2, $NH(C_{1-3}$alkyl), $N(C_{1-3}$alkyl$)_2$, CN, NO_2, aryl or heteroaryl;

R_{27} is hydroxy, C_{1-6}alkoxy, SH, SC_{1-6}alkyl, halo, NH_2, $NH(C_{1-3}$alkyl), $N(C_{1-3}$alkyl$)_2$, $C(O)R_{31}$, aryl or heterocyclyl;

Each R_{28} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl or $(CR_{26}R_{26'})_nR_{30}$;

R_{29} is the characterising group of an amino acid;

R_{30} is halogen, hydroxy, C_{1-3}alkoxy, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, $C(O)R_{31}$, aryl or heterocyclyl;

R_{31} is C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl or heterocyclyloxy;

q is 0, 1, 2 or 3;

n is 0, 1, 2 or 3;

m is 0 or 1 to 20;

r is 1 to 5;

s is 1 to 10; and

t is 1 or 2;

wherein an alkyl, alkenyl, alkynyl, alkyloxy, aryl or heterocyclyl group may be optionally substituted one or more times.

The present inventors have shown in PCT/AU02/00717 that compounds of general formula I are inhibitors of the cytokine or biological activity of MIF. The present inventors have also shown in PCT/AU02/00716 that compounds of general formula II are inhibitors of the cytokine or biological activity of MIF.

In a second aspect, the present invention provides a method for inhibiting the cytokine or biological activity of MIF in a subject comprising the step of implanting a device according to the first aspect in the subject.

Preferably, the method is for inhibiting the cytokine or biological activity of MIF in a local region of the subject and the device is implanted within or proximate to the local region of the subject.

In a third aspect, the present invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine activity is implicated comprising the step of implanting a device according to the first aspect in a subject in need thereof.

Preferably, the disease or condition is confined to a local region of the subject and the device is implanted within or proximate to the local region.

Preferably the disease or condition is selected from autoimmune diseases, diseases characterised by cell proliferation, solid or haemopoeitic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Vascular diseases (including but not limited to neointimal hyperplasia, vascular stent restenosis, neo-angiogenesis, diabetic retinopathy), atherosclerosis (eg ischaemic heart disease, myocardial infarction, cerebrovascular disease, stroke, peripheral vascular disease, atherosclerotic plaque rupture), rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis, polymyalgia rheumatica) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, interstitial nephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg dementia, Alzheimer's disease, multiple sclerosis, demyelinating diseases), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, parturition, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis,

UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing.

More preferably, the disease or condition is selected from autoimmune diseases, diseases characterised by cell proliferation, solid or haemopoeitic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Vascular diseases (including but not limited to neointimal hyperplasia, vascular stent restenosis, neo-angiogenesis, diabetic retinopathy), atherosclerosis (eg ischaemic heart disease, myocardial infarction, cerebrovascular disease, stroke, peripheral vascular disease, atherosclerotic plaque rupture), rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer, multiple myeloma and metastatic cancer), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, parturition, endometriosis), psoriasis, pelvic inflammatory disease, transplant rejection, allergics, allergic rhinitis, atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), pain, inflammatory consequences of trauma or ischaemia, and wound healing.

Yet more preferably, the disease is selected from autoimmune diseases, diseases characterised by cell proliferation, solid or haemopoeitic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Vascular diseases (including but not limited to neointimal hyperplasia, vascular stent restenosis, neo-angiogenesis, diabetic retinopathy), atherosclerosis (eg ischaemic heart disease, myocardial infarction, cerebrovascular disease, stroke, peripheral vascular disease, atherosclerotic plaque rupture), rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) inflammatory

bowel disease (including but not limited to ulcerative colitis, Crohn's disease), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, asthma, bronchitis, bronchiectasis, chronic obstructive pulmonary disease,), corneal disease, iritis, iridocyclitis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, parturition, endometriosis), psoriasis, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), pain, inflammatory consequences of trauma or ischaemia, and wound healing.

Even more preferably, the disease or condition is selected from autoimmune diseases, diseases characterised by cell proliferation, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Vascular diseases (including but not limited to neointimal hyperplasia, vascular stent restenosis, neo-angiogenesis, diabetic retinopathy), atherosclerosis (eg ischaemic heart disease, myocardial infarction, cerebrovascular disease, stroke, peripheral vascular disease, atherosclerotic plaque rupture), rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), pain, and wound healing.

The inventors have shown that compounds that act as MIF inhibitors are effective in the treatment of atheroma. It will be appreciated by those skilled in the art that demonstration of a beneficial effect of a MIF antagonist on the biological processes operative in atheroma may be consistent with a possible beneficial effect on other vascular diseases characterised by similar cellular processes, or are influenced by the presence of atheroma, such as stent restenosis.

Accordingly, in a third aspect, the present invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine activity is implicated comprising the step of implanting a device according to the first aspect in a subject in need thereof.

Preferably, the disease or condition is confined to a local region of the subject and the device is implanted within or proximate to the local region.

In an alternative embodiment, the disease or condition is a systemic disease or condition. In this embodiment, the implantable device is used to administer the MIF inhibitor systemically.

In a fourth aspect the present invention provides an angioplastic stent operably coated with a prophylactically effective dose of a composition comprising at least one MIF inhibitor.

The term "prophylatically effective dose" means an amount sufficient to inhibit or prevent the onset of restensosis in the vicinity of the stent.

Angioplastic stents, also known by other terms such as "intravascular stents" or simple "stents", are well known in the art. They are routinely used to prevent vascular closure due to physical anomalies such as unwanted inward growth of vascular tissue due to surgical trauma. They often have a tubular, expanding lattice-type structure appropriate for their function, and can optionally be biodegradable.

In this invention, the stent can be operably coated with at least one MIF inhibitor using any suitable means known in the art. Here, "operably coating" a stent means coating it in a way that permits the timely release of the MIF inhibitor(s) into the surrounding tissue to be treated once the coated stent is administered. Such coating methods, for example, can use the polymer polypyrrole or phosphorylcholine.

In a fifth aspect, the present invention provides a method for inhibiting the onset of restenosis in a subject undergoing angioplasty, which comprises topically administering a stent according to the fourth aspect to the subject at around the time of the angioplasty.

As used herein, administration "at around the time of angioplasty" can be performed during the procedure, or immediately before or after the procedure. The administering can be performed according to known methods such as catheter delivery.

In a sixth aspect, there is provided a method of reducing the severity of stent restenosis in the vicinity of a stent comprising the use of a stent according to the fifth aspect.

The construction of stents that release or elute a pharmaceutical active is known to those skilled in the art. The standard approach is to use current highly refined metallic stent designs with polymer materials that release the active in a controlled manner. Several polymer materials have been used for the coating of stents to permit the elution of drugs. These include biocrodible polymers such as poly-L lactic acid, biostable polymers such as polyurethane derivatives and silicone-based polymers, as well as hydrogels. It will be

recognised by those skilled in the art that the function of a drug-eluting stent requires the drug to be bound to the stent or its polymer or other coating in such a way as to allow steady release of drug over a period of time, and that the drug is able to be locally absorbed into cells in the vessel and stent lumen. The optimum stent coating material and delivery parameters vary according to the tissue retention of the drug, such that rapid release of a tissue-retained drug can have long lasting effects, whereas a drug retained in tissues for a shorter time would need to be released over a longer period. A person skilled in the art would be able to select appropriate materials and conformations of stent for a particular purpose and particular small molecule inhibitor.

Examples of commercially available stents which may be adapted by a person skilled in the art for use in the present invention include:

Cypher® Sirolimus-eluting Stent (Cordis)

TAXUS™ Express2™ Tacrolimus eluting stent (Boston Scientific Corporation)

Express2 bare metal stent (Boston Scientific Corporation)

Medtronic® Vascular S660 Coronary Stent (Medtronic)

Aspire® Covered Stent (Vascular Architects, Inc)

COSTAR® (cobalt chromium paclitaxel drug eluting stent (BIOTRONIK AG)

Dexamet® (Abbott)

BiodivYsio coronary stent (Abbott/Biocompatibles)

SCIMED® RADIUS Coronary Stent

NIRflex™ Pre-Mounted Coronary Stent (Medinol Ltd)

BeStent™ 2 (Medtronic)

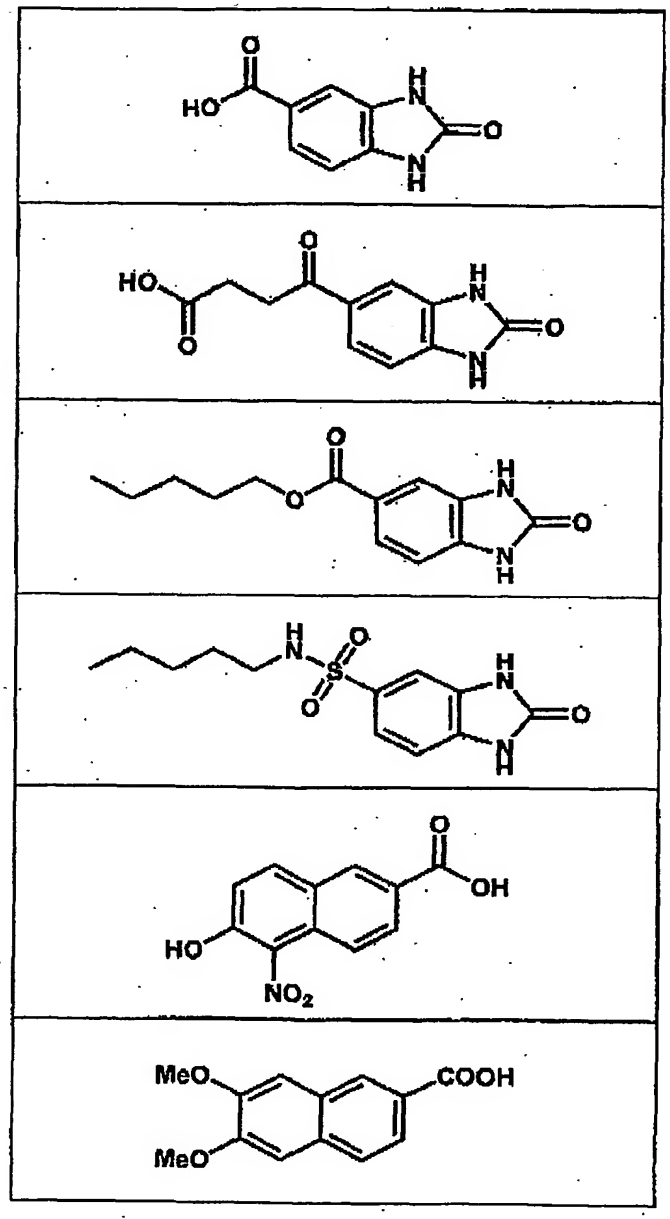
MULTI-LINK VISION™ RX & OTW Coronary Stent System (Guidant Corporation)

ACCULINK™ Carotid Stent System (Guidant Corporation)

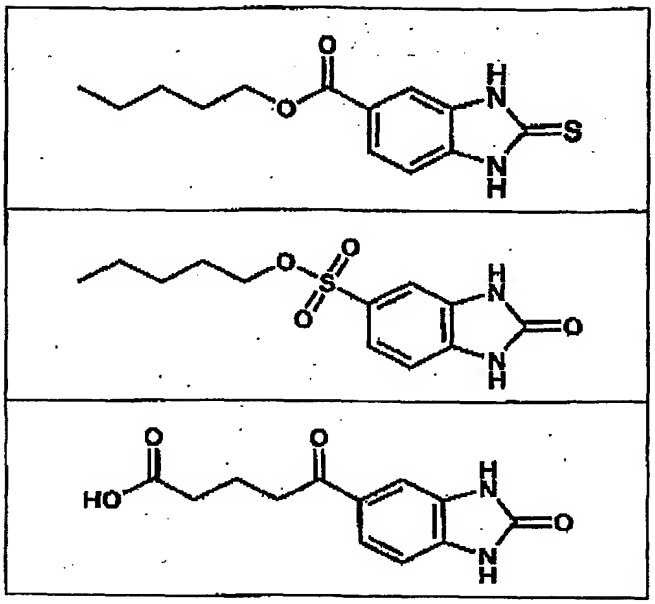
S.M.A.R.T.™ Nitinol Stent System (Cordis)

Preferably, the MIF inhibitor is selected from the compounds of Table 1.

Table 1



19.

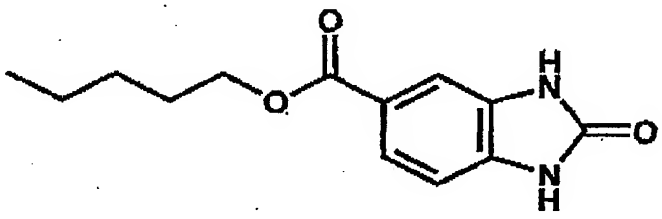


In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Experiments were carried out using pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate, the compound of formula III:



III

METHODS

C57BL/6J mice homozygous for the disrupted ApoE gene (ApoE-/-) were obtained from

Jackson Laboratories. They were maintained under standard conditions with food and water available *ad libitum* 8 weeks of age. All male mice were then used in the studies from 8-20 weeks of age. From 8 weeks of age the mice were fed a Western diet *ad libitum*, also known as "High Fat Diet" (HFD) comprising of 21% butter, 0.15% cholesterol (obtained from Specialty Feeds).

III was dissolved in a vehicle comprised of 75µl of Dimethyl Sulfoxide (DMSO) and 25µl vegetable oil. III (15 mg/kg/day) or vehicle were administered daily by oral gavage to ApoE-/- mice fed a high fat diet. Treatment was commenced when the mice reached 8 weeks of age and continued until 16 weeks of age. At this point mice were killed, peripheral blood collected for analysis of cholesterol concentration, and the aorta collected for morphological studies on atherosclerotic lesions.

The thoracic aorta was dissected from the mice and the most proximal segment containing the aortic valve specifically analysed. Tissues for RNA analysis were snap frozen in liquid nitrogen and stored at - 80°C. Tissues for morphology and immunohistochemistry were frozen in compound embedding medium OCT, and the embedded tissue was stored at - 80°C.

RNA was extracted from tissue samples pooled from 2-4 mice using Solution D (comprising 25g Guanidine, 29ml RNase free water, 1.95 ml Sodium citrate, and 2.64 ml 10% Sarkosyl). The presence of MIF mRNA in the atherosclerotic lesion was assessed using RT-PCR (Invitrogen one step RT-PCR kit), The RT-PCR reactions were carried out in a 25µl volume containing: 12.5 2X reaction mix, and 1µl of primer mix for a final concentration of 0.2µM, 0.5µl of RT platinum Taq mix, 1µl of RNA sample and 10µl of RNase free H2O. RT-PCR was also used to assess GAPDH mRNA expression in the total RNA samples. Control reactions included those in which no reverse transcription was performed on the RNA samples. Primers used were as follows: MIF: Sense: 5' CAG CGC GCT TTG TAC CGT CCT C 3'; anti-sense: 5' CGT TGG CAG CGT TCA TGT CGT AAT AGT T 3' GAPDH; Sense 5' ATG TTT GTG ATG GGT GTG AAC CAG G 3'; anti-sense: 5'TAG CCA TAT TCA TTG TCA TAC CAG G 3'. The PCR products were resolved by agarose gel electrophoresis with ΦcX174/HaeIII (Promega) as size standards. cDNA fragments were visualized under UV light and photographed.

Frozen tissue was cut at 6µm using a Leica Cryostat, and collected on Super Frost Plus slides (Menzel Glaser). The lipid content of the atherosclerotic lesions, and their size, was assessed by Oil Red-O staining, counterstained with Meyer's hematoxylin. Immunohistochemical

techniques were used to identify MIF, and macrophage and T cell markers, in aortic lesions.

RESULTS

ApoE -/- mice fed a HFD developed significant atherosclerotic vascular disease, characterised by lesions with substantial Oil Red-O staining, and macrophage and T cell infiltrate.

In order to verify that MIF was present in mouse atheroma, semi-quantitative RT-PCR was performed on samples from ApoE -/- mice exposed to high fat diet but not treated with III or vehicle. The PCR product was run on a 2% agarose gel and the results are shown in Figure 1. Figure 1 shows that MIF mRNA is expressed in the aorta of mice with atheromatous lesions.

MIF expression in lesions of mice fed HFD was confirmed using immunohistochemistry. MIF immunostaining was significantly increased at times points after 4 week HFD treatment (Figure 2) ($p < 0.001$). These data show that an increase in aortic MIF expression is associated with atheroma development and progression. It will be appreciated by those skilled in the art that expression of MIF within these vascular lesions is consistent with the hypothesis that suppression of MIF could exert a beneficial effect on these lesions.

Sections were collected from mice treated with III 15 mg/kg/24h from week 8-16 by once daily oral gavage, and stained with Oil Red-O to determine lesion size. As shown in Figure 3, the measured total lesion area in III-treated mice was significantly lower than those treated with MIF vehicle ($p = 0.002$). It will be appreciated by those skilled in the art that the effect of MIF antagonism was statistically significant, and of a substantial magnitude, consistent with a beneficial effect of III on the prevention of atheroma.

Atheroma lesion severity was further analysed by analysis of macrophage numbers. Macrophage staining in atheroma lesions was determined using CD68, as shown in Figure 4. The mean CD68+ cell count in atheroma lesions was significantly lower in mice receiving III 15 mg/kg/24h from week 8-16 by once daily oral gavage ($p=0.022$). It will be appreciated by those skilled in the art that the effect of MIF antagonism was statistically significant, and of a substantial magnitude, consistent with a beneficial effect of III on the prevention of atheroma.

To ensure that the effects of III were not due to effects on plasma cholesterol, plasma cholesterol levels were measured and are shown in Figure 5. No effect on plasma cholesterol

was observed with III administration at the dose used to exert an inhibitory effect on atheroma progression.

EXAMPLE 2

Studies suggest that stent restenosis is largely a result of vascular smooth muscle cell (VSMC) proliferation, which is exaggerated after stent deployment due to the high pressure technique of stent deployment. The study reported below was conducted in order to assess the effects of MIF and of MIF inhibitors on the proliferation of vascular smooth muscle cells.

Example 2(a)

MIF-induced mouse vascular smooth muscle cell proliferation

Methods

The effects of MIF treatment on the survival and proliferation of primary mouse vascular smooth muscle cells (VSMC) were tested. These cells were isolated from mouse aortas using standard techniques [Ray JL, Leach R, Herbert J-M, Benson M "Isolation of smooth muscle cells from a single murine aorta" Methods in Cell Science 23, 185-188, 2002]. Briefly, mouse aortae were collected, cleaned of fat and adventitia, and cut into small (approximately 1mm square) pieces. Pieces were placed into 300µl of enzyme solution, prepared by dissolving 11 mg of type II collagenase and 2.75 mg elastase in 5.5 ml of Dulbecco's modified Eagle's medium (DMEM), and incubated for 4-6 hours at 37°C with gentle shaking. Cell suspensions were washed in medium and cells plated onto 30 mm tissue culture plates in DMEM with 15-20 % foetal bovine serum (FBS).

The cells were maintained in DMEM supplemented with 10% FBS. Prior to experimentation, cells were seeded at 10^3 cells/well in a 96-well plate in DMEM/10% FBS. After 4 hr cells were transferred to DMEM/0.5% FBS and incubated for 18hr prior to treatment for 48 hr with DMEM-containing either 0.5% or 10% FBS plus 0, 50, 200 or 500 ng/ml of recombinant *E. coli*-derived human MIF protein. As a readout of cellular proliferation, total cellular ATP levels in each well were determined by addition of CellTiter Glo Reagent (Promega) as described by the manufacturer. The resulting relative luminescent unit (RLU) values were measured using a Packard Lumi Count Microplate Luminometer. Total cellular ATP levels are directly proportional to viable cell numbers.

Significant changes in total cellular ATP levels were determined by the demonstration of a significant P value ($P < 0.05$) using an unpaired t-test.

Results

Treatment of VSMC with 10% FBS induces a significant increase in total cellular ATP pool levels as determined by the increase in RLU value relative to 0.5% FBS (Figure 6) ($P<0.001$). Total cellular ATP levels are directly proportional to viable cell numbers and the above result therefore shows that the viability and proliferation of VSMC is enhanced by 10% FBS.

The addition of 50 and 200 ng/ml of MIF protein in the presence of 10% FBS resulted in a further significant increase in total cellular ATP levels (Figure 7) ($P<0.05$). These data are therefore consistent with a role for MIF in the survival and proliferation of VSMC.

Example 2(b)

Detection of MIF protein in vascular smooth muscle cell lysates and conditioned media

To ensure the presence of MIF in the experimental conditions in which MIF antagonist compounds were tested, MIF concentration was measured in culture supernatants and cell lysates of murine vascular smooth muscle cells (VSMC).

Methods

Semi-confluent cultures of VSMC in 10 cm plates were cultured for 72 hr with DMEM containing either 10% or 0.5% fetal bovine serum (FBS). The resulting conditioned medium was then collected for analysis of MIF protein levels by enzyme-linked immunosorbent assay (ELISA) using antibodies from R&D Systems (Minneapolis, USA) according to the manufacturer's instructions. The VSMC incubated in the presence 10% FBS were also directly lysed in buffer containing 20 mM Hepes, pH 7.2, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 20 mM beta-glycerophosphate, 100 mM NaCl, 0.05% Triton X100, 0.5 mM DTT, 20 ug/ml leupeptin, 100 ug/ml PMSF and 20 ug/ml aprotinin. The cell lysates were then assayed for MIF protein by ELISA.

Results

The conditioned media resulting from culture with the VMSC contains detectable levels of MIF, which are highest in the cells cultured in 10% FBS. Significant levels of MIF protein was also detected in the VSMC cellular lysates. These results demonstrate constitutive production and release of MIF by cultured VSMC (see Figure 8).

Inhibition of mouse vascular smooth muscle cell proliferation by compounds.

Methods

The effects of compound treatment on mouse vascular smooth muscle cell (VSMC) survival and proliferation were tested. These cells were seeded at 10^3 cells/well in a 96-well plate in DMEM/10% FBS. After 4 hr cells were transferred to DMEM/0.5% FBS and incubated for 18hr prior to treatment for 48 hr with DMEM-containing either 0.5% FBS or 10% FBS plus a MIF inhibitor of the invention at a concentration of 0.1, 1, 10 or 100 µM. Total cellular ATP levels in each well were determined by addition of CellTiter-Glo Reagent (Promega) as described by the manufacturer. The resulting relative RLU values were measured using a Packard Lumi Count Microplate Luminometer. For each compound the maximal inhibition of total cellular ATP levels was expressed as a percent inhibition of the difference between the values determined for 10% FBS versus 0.5% FBS (see Table 2).

Significant changes in total cellular ATP levels were determined by the demonstration of a significant P value using an unpaired t-test.

Results

Treatment with compound pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate, when used in the method above, significantly inhibited VSMC survival and proliferation ($P < 0.001$), as shown in Table 2 and Figure 9. This effect approached the effect of culturing cells in 0.5% FBS, as shown. These effects of pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate are therefore consistent with a significant inhibition of VSMC proliferation.

Treatment with compound 2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide when used in the method above, significantly inhibited vascular smooth muscle survival and proliferation ($P < 0.001$), as shown in Table 2 and Figure 10. This effect exceeded the effect of culturing cells in 0.5% FBS, as shown. These effects of 2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide are therefore consistent with a significant inhibition of vascular smooth muscle cell proliferation.

Treatment with the following compounds, 6-hydroxy-5-nitro-2-naphthoic acid, 4-oxo-4-(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)butanoic acid, 4-{[(2-oxo-2,3-dihydro-1H-indol-5-yl)sulfonyl]amino}butanoic acid, 6,7-dimethoxy-2-naphthoic acid, 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylic acid, 6-oxo-6-(2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)hexanoic acid, 4-{[(2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)sulfonyl]amino}butanoic acid, and 1-

methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylic acid also induced inhibition of the survival and proliferation of the vascular smooth muscle cells (see Table 2). These data are consistent with these compounds exerting inhibitory effects on vascular smooth muscle cell proliferation by inhibition of MIF.

Table 2 – Inhibition of VSMC proliferation by MIF antagonist compounds

Compound	% inhibition of VSMC proliferation relative to low serum*	Compound Concentration (μM)
2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide	132.6	100
pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate	83.8	100
6-hydroxy-5-nitro-2-naphthoic acid	45.9	100
4-oxo-4-(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)butanoic acid	37.8	10
6,7-dimethoxy-2-naphthoic acid	30.0	100
2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylic acid	30.0	1
6-oxo-6-(2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)hexanoic acid	19.5	0.1
4-{[(2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)sulfonyl]amino}butanoic acid	17.7	100
1-methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylic acid	12.9	100

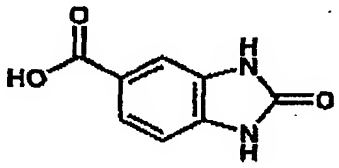
* mean of 1-3 experiments, each performed in triplicate.

EXAMPLE 3

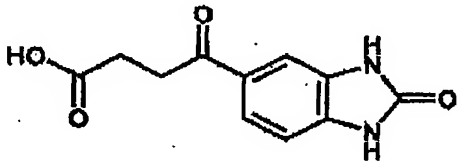
Loading and Release of Compounds from Stents

The following procedures were used to investigate the loading and release of compounds from Bio*div Ysio*™ phosphorylcholine coated stainless steel coronary stents made by Biocompatibles Ltd.

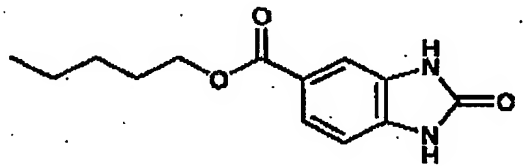
Compounds Used



2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylic acid (1)

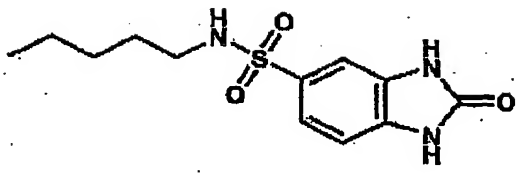


4-oxo-4-(2-oxo-2,3-dihydro-1H-1,3-benzimidazol-5-yl)butanoic acid (2)

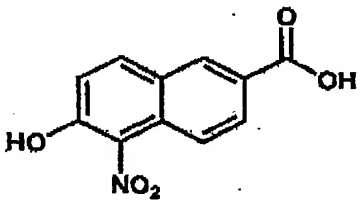


pentyl 2-oxo-2,3-dihydro-1H-1,3-benzimidazole-5-carboxylate (3)

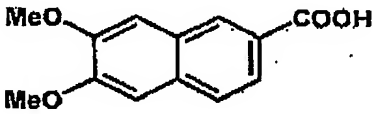
27.



2-oxo-N-pentyl-2,3-dihydro-1H-1,3-benzimidazole-5-sulfonamide (4)



6-hydroxy-5-nitro-2-naphthoic acid (5)



6,7-dimethoxy-2-naphthoic acid (6)

Compound Loading

Solutions of the compounds were made in either *N,N*-dimethylformamide (DMF) or methanol as shown in Table 1. A phosphorylcholine coated stent was immersed in each solution for 5 minutes then withdrawn and allowed to dry (DMF was removed by evaporation under high vacuum). Additional compound was loaded onto each stent by applying a 10 µl drop of the appropriate solution to the stent and re-drying. This additional loading was performed twice for each of the stents.

Table 3

Compound	Solution conc (mg/ml)	Solvent	Nominal Stent Length (mm)	Nominal Stent Diameter (mm)
1	11	DMF	11	3.0
2	22	DMF	11	3.0
3	6.7	methanol	28	3.5
4	6.7	methanol	28	3.5
5	5.2	methanol	18	3.0
6	5.5	methanol	18	3.0

Compound Release

Each dried phosphorylcholine coated stents was totally immersed in an accurately known volume of phosphate-buffered saline solution (PBS-26.96 g/L Na_2HPO_4, 1.56 g/L NaH_2PO_4, 8.5 g/L NaCl-pH 7.2) of between 2-3 ml (see Table 4), which was agitated by a small magnetic stirring bar at room temperature. An aliquot of the solution was taken at regular intervals and its absorbance at an appropriate wavelength (λ_{max}) for the compound was measured before being returned to the flask containing the stent.

Table 4

Compound	PBS volume (ml)	wavelength monitored	95% release (min)
1	2.0	275 nm	24
2	2.0	330 nm	35
3	2.8	266 nm	91
4	3.0	287 nm	94
5	3.0	435 nm	13
6	3.0	286 nm	5

Standard curves of compound concentration versus absorbance were prepared for each compound using PBS as a reference, allowing the concentration of the compounds at the different time-points to be determined. Multiplication of the concentration in µg/ml by the PBS volume in ml gives the total mass in µg of compound released at that time-point. Mass release profiles, Figures 11 and 12 for compounds 3 and 4 respectively, were generated by plotting the mass released versus time. The time required to release 95% of the loaded mass is given in Table 4 for each compound.

Conclusion

Examination of the mass release profiles for compounds 1-6 show that between 100 and 245 µg of material was able to be loaded onto the stent successfully, then released into phosphate buffered saline over a 15-200 minute time period.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present

invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS

1. An implantable device comprising:

   (i) a reservoir containing at least one MIF inhibitor; and

   (ii) means to release or elute the inhibitor from the reservoir

2. A method for inhibiting the cytokine or biological activity of MIF in a subject comprising the step of implanting a device according to claim 1.

3. A method according to claim 2 wherein the method is for inhibiting the cytokine or biological activity of MIF in a local region of the subject and the device is implanted within or proximate to the local region of the subject.

4. A method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine activity is implicated comprising the step of implanting a device according to claim 1 in a subject in need thereof.

5. A method according to claim 4 wherein the disease or condition is confined to a local region of the subject and the device is implanted within or proximate to the local region.

6. A method according to claim 4 wherein the disease or condition is a systemic disease or condition.

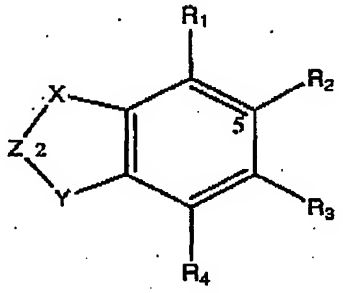
7. An angioplastic stent operably coated with a prophylactically effective dose of a composition comprising at least one MIF inhibitor.

8. A method for inhibiting the onset of restenosis in a subject undergoing angioplasty, which comprises topically administering a stent according to claim 7 to the subject at around the time of the angioplasty.

9. A method of inhibiting vascular smooth muscle proliferation in a subject which comprises topically administering a stent according to claim 7 to the subject.

10. A method of reducing the severity of stent restenosis in the vicinity of a stent comprising the use of a stent according to claim 7.

11. A device according to claim 1 or a stent according to claim 7 wherein the MIF inhibitor is a compound of the general formula (I) or (II), or a pharmaceutically acceptable salt or prodrug thereof:



I

wherein

X is selected from -O-, -S-, -C(R_5)($R_{5'}$)- or -N(R_6)- and preferably comprises a hydrogen bond donor or acceptor;

Y is selected from -N(R_7)-, -O-, -S- or -C(R_7)$_2$-;

Z is selected from -C(O)-, -C(S)-, -C(=NR_6)-, -S(O)- or -S(O)$_2$-;

R_1 is selected from hydrogen, C_{1-3}alkyl, ($CR_5R_{5'}$)$_n$OR_7, ($CR_5R_{5'}$)$_n$SR_7, ($CR_5R_{5'}$)$_n$N(R_6)$_2$ and ($CR_5R_{5'}$)$_n$halo;

R_2 is selected from C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, ($CR_{12}R_{12'}$)$_m$C(O)R_8, ($CR_{12}R_{12'}$)$_m$C(S)R_8, ($CR_{12}R_{12'}$)$_m$S(O)R_8, ($CR_{12}R_{12'}$)$_m$S(O)$_2R_8$, ($CR_{12}R_{12'}$)$_m$OR_9, ($CR_{12}R_{12'}$)$_m$SR_9, ($CR_{12}R_{12'}$)$_m$N$R_{10}R_{11}$, ($CR_{12}R_{12'}$)$_m$C(=NR_{24})R_{22} and ($CR_{12}R_{12'}$)$_m$$R_{13}$;

R_3 is selected from hydrogen, C_1-C_6alkyl, ($CR_{16}R_{16'}$)$_p$N$R_{14}R_{15}$, ($CR_{16}R_{16'}$)$_p$OR_{17}, ($CR_{16}R_{16'}$)$_p$SR_{17}, ($CR_{16}R_{16'}$)$_p$halo, ($CR_{16}R_{16'}$)$_p$NO_2, ($CR_{16}R_{16'}$)$_n$C(O)R_{28}, ($CR_{16}R_{16'}$)$_n$C(=NR_{24})R_{22}, ($CR_{16}R_{16'}$)$_n$S(O)R_{17}, ($CR_{16}R_{16'}$)$_n$S(O)$_2R_{17}$, ($CR_{16}R_{16'}$)$_n$S(O)$_3R_{17}$ and ($CR_{16}R_{16'}$)$_p$C(R_{18})$_3$;

R_4 is selected from hydrogen, halogen C_1-C_3alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl and ($CR_{12}R_{12'}$)$_n$C(R_{18})$_3$;

Each R_5 and $R_{5'}$ is independently selected from hydrogen, C_1-C_3alkyl, halo, OR_7, SR_7 and $N(R_6)_2$;

Each R_6 is independently selected from hydrogen, C_1-C_3alkyl and OR_7;

Each R_7 is independently selected from hydrogen and C_1-C_3alkyl;

R_8 is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, OR_{19}, SR_{19}, $N(R_{20})_2$, $[NH\text{-}CH(R_{21})\text{-}C(O)]_q\text{-}OR_{29}$, $[sugar]_q$ and $(CR_{12}R_{12'})_tR_{13}$;

R_9 is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, $(CR_{12}R_{12'})_tR_{13}$, $C(O)R_{23}$, CO_2R_{23}, $C(S)R_{23}$, $C(S)OR_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q\text{-}R_{23}$ and $[sugar]_q$;

R_{10} and R_{11} are independently selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, $(CR_{12}R_{12'})_mR_{13}$, $C(O)R_{23}$, $C(S)R_{23}$, $S(O)R_{23}$, $S(O)_2R_{23}$, $[C(O)CH(R_{21})NH]_q\text{-}R_{23}$, $\text{-}[sugar]_q$ and $NHC(=NR_{25})\text{-}NH_2$;

Each R_{12} and $R_{12'}$ is independently selected from hydrogen, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, OR_{24}, SR_{24}, halo, $N(R_{24})_2$, CO_2R_{24}, CN, NO_2, aryl or heterocyclyl;

R_{13} is selected from OR_{25}, SR_{25}, halo, $N(R_{25})_2$, $C(O)R_{31}$, CN, $C(R_{18})_3$, aryl or heterocyclyl;

R_{14} and R_{15} are independently selected from hydrogen, C_1-C_3alkyl, OR_{17}, $(CR_{16}R_{16'})_pC(R_{18})_3$;

Each R_{16} and $R_{16'}$ is independently selected from hydrogen, C_1-C_3alkyl, halo, OR_{17}, SR_{17} and $N(R_{17})_2$;

Each R_{17} is independently selected from hydrogen and C_1-C_3alkyl;

Each R_{18} is independently selected from hydrogen and halo;

R_{19} and each R_{20} are independently selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, $(CR_{26}R_{26'})_tR_{27}$;

R_{21} is the characterising group of an amino acid;

R_{22} is selected from C_1-C_6alkyl, NH_2, $NH(C_{1\text{-}6}alkyl)$, $N(C_{1\text{-}6}alkyl)_2$, OR_{29} or SR_{29};

R_{23} is selected from hydrogen, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, aryl $(CR_{26}R_{26'})_tR_{27}$;

Each R_{24} is independently selected from hydrogen and C_1-C_6alkyl;

Each R_{25} is independently selected from hydrogen, C_1-C_6alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aryl and heterocyclyl;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_1-C_6alkyl, C_2-C_6alkenyl, C_2-C_6alkynyl, OR_{29}, SR_{29}, halo, $N(R_{29})_2$, CO_2R_{29}, CN, NO_2, aryl and heterocyclyl;

R_{27} is selected from hydrogen, OR_{30}, SR_{30}, halo, $N(R_{30})_2$, CO_2R_{30}, aryl and heterocyclyl;

R_{28} is selected from hydrogen, C_{1-6}alkyl, OR_{29}, SR_{29} or $N(R_{29})_2$;

Each R_{29} is independently selected from hydrogen and C_1-C_3alkyl;

Each R_{30} is independently selected from hydrogen, C_1-C_3alkyl, aryl and heterocyclyl;

R_{31} is selected from C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

n is 0 or an integer from 1 to 3;

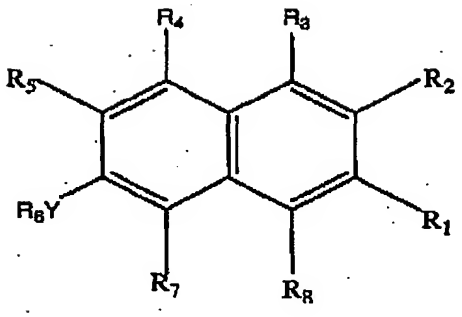
m is 0 or an integer from 1 to 20;

p is 0 or an integer from 1 to 6;

q is an integer from 1 to 5;

t is an integer from 1 to 10;

wherein alkyl, alkenyl, alkynyl, aryl and heterocyclyl may be optionally substituted; and



II

wherein

Y is O, NR_9 or $S(O)_q$,

R_1 is selected from hydrogen, C_{1-6}alkyl, $-(CR_{10}R_{10'})_n$halo, $-(CR_{10}R_{10'})_nOR_{11}$, $-(CR_{10}R_{10'})_n-SR_{11}$, $-(CR_{10}R_{10'})_n-N(R_{12})_2$, $-(CR_{10}R_{10'})_nS(O)R_{11}$, $-(CR_{10}R_{10'})_nS(O)_2R_{11}$, $-(CR_{10}R_{10'})_n-S(O)_3R_{11}$, $-(CR_{10}R_{10'})_nC(O)R_{13}$, $-(CR_{10}R_{10'})_n-C(=NR_{14})R_{15}$ or $-(CR_{10}R_{10'})_nR_{16}$;

R_2 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $-(CR_{10}R_{10'})_mOR_{17}$, $-(CR_{10}R_{10'})_mSR_{17}$, $-(CR_{10}R_{10'})_mNR_{18}R_{19}$, $-(CR_{10}R_{10'})_mS(O)R_{20}$, $-(CR_{10}R_{10'})_mS(O)_2R_{20}$, $-(CR_{10}R_{10'})_mC(O)R_{20}$, $-(CR_{10}R_{10'})_mC(S)R_{20}$, $-(CR_{10}R_{10'})_mC(=NR_{11})R_{15}$ or $-(CR_{10}R_{10'})_mR_{16}$;

R_3, R_4 and R_5 are independently selected from hydrogen, C_{1-3}alkyl, $-(CR_{10}R_{10'})_nN(R_{14})_2$, $-(CR_{10}R_{10'})_nOR_{14}$, $-(CR_{10}R_{10'})_nSR_{14}$ or $-(CR_{10}R_{10'})_n$halo;

R_6 is selected from hydrogen, C_{1-6}alkyl, $-C(O)C_{1-6}$alkyl, $-C(O)N(R_9)_2$-, $-C(S)N(R_9)_2$- or $-(CR_{10}R_{10'})_nR_{21}$, or R_6Y and R_5 together may form $-X-(CH_2)_t-Z-$, where X and Z may be independently selected from O, S or NR_{14};

R_7 and R_8 are independently selected from hydrogen, C_{1-3}alkyl, C_{2-3}alkenyl, C_{2-3}alkynyl or $-(CR_{10}R_{10'})_nR_{22}$;

Each R_9 is independently selected from hydrogen or C_{1-6}alkyl;

Each R_{10} and $R_{10'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, OR_{11}, SR_{11}, C_{1-3}alkoxy, CO_2R_{14}, $N(R_{14})_2$, CN,

NO_2, aryl or heterocyclyl;

R_{11} is hydrogen or C_{1-6}alkyl;

Each R_{12} is independently selected from hydrogen, C_{1-6}alkyl, $C(=NR_{14})R_{15}$, $NH\text{-}C(=NR_{14})R_{15}$, $C(O)R_{14}$ or $C(S)R_{14}$;

R_{13} is hydrogen, C_{1-6}alkyl, OR_{14}, SR_{14} or $N(R_{14})_2$;

Each R_{14} is independently selected from hydrogen or C_{1-3}alkyl;

R_{15} is C_{1-6}alkyl, NH_2, $NH(C_{1-3}alkyl)$ or $N(C_{1-3}alkyl)_2$, OR_{23} or SR_{23};

R_{16} is hydroxy, C_{1-3}alkoxy, SH, SC_{1-3}alkyl, halo, $C(O)R_{31}$, $C(R_{24})_3$, CN, aryl or heterocyclyl;

R_{17} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, CO_2R_{25}, $C(S)R_{25}$, $C(S)OR_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r\text{-}R_{23}$ or $[sugar]_r$;

R_{18} and R_{19} are independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(CR_{26}R_{26'})_sR_{27}$, $C(O)R_{25}$, $C(S)R_{25}$, $S(O)R_{25}$, $S(O)_2R_{25}$, $[C(O)CH(R_{29})NH]_r\text{-}R_{23}$, $[sugar]_r$, $C(=NR_{23})NH_2$ or $NH\text{-}C(=NR_{23})NH_2$;

R_{20} is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OR_{28}, SR_{28}, $N(R_{28})_2$, $[NH\text{-}CHR_{29}C(O)]_r\text{-}OR_{23}$, $[sugar]_r$, or $(CR_{26}R_{26'})_sR_{27}$;

R_{21} is OR_{28}, SR_{28}, halo or $N(R_{25})_2$;

R_{22} is halo, CO_2H, SO_3H, NO_2, NH_2, CO_2C_{1-3}alkyl, SO_3C_{1-3}alkyl or $C(R_{24})_3$;

R_{23} is hydrogen or C_{1-3}alkyl;

Each R_{24} is independently selected from hydrogen, Cl or F;

Each R_{25} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, aryl or $(CR_{26}R_{26'})_sR_{27}$;

Each R_{26} and $R_{26'}$ is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, hydroxy, C_{1-3}alkoxy, SH, C_{1-3}alkylthio, CO_2H, CO_2C_{1-3}alkyl, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, CN, NO_2, aryl or heteroaryl;

R_{27} is hydroxy, C_{1-6}alkoxy, SH, SC_{1-6}alkyl, halo, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, $C(O)R_{31}$, aryl or heterocyclyl;

Each R_{28} is independently selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl or $(CR_{26}R_{25'})_sR_{30}$;

R_{29} is the characterising group of an amino acid;

R_{30} is halogen, hydroxy, C_{1-3}alkoxy, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, $C(O)R_{31}$, aryl or heterocyclyl;

R_{31} is C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl or heterocyclyloxy;

q is 0, 1, 2 or 3;

n is 0, 1, 2 or 3;

m is 0 or 1 to 20;

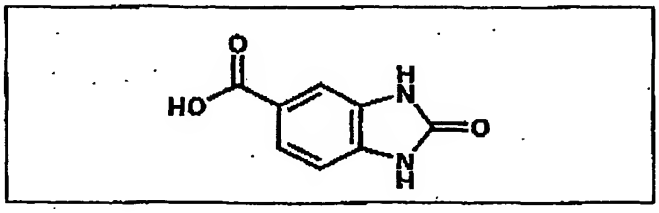
r is 1 to 5;

s is 1 to 10; and

t is 1 or 2;

wherein an alkyl, alkenyl, alkynyl, alkyloxy, aryl or heterocyclyl group may be optionally substituted one or more times.

12. A device or stent according to claim 11 wherein the MIF inhibitor is selected from the group consisting of:



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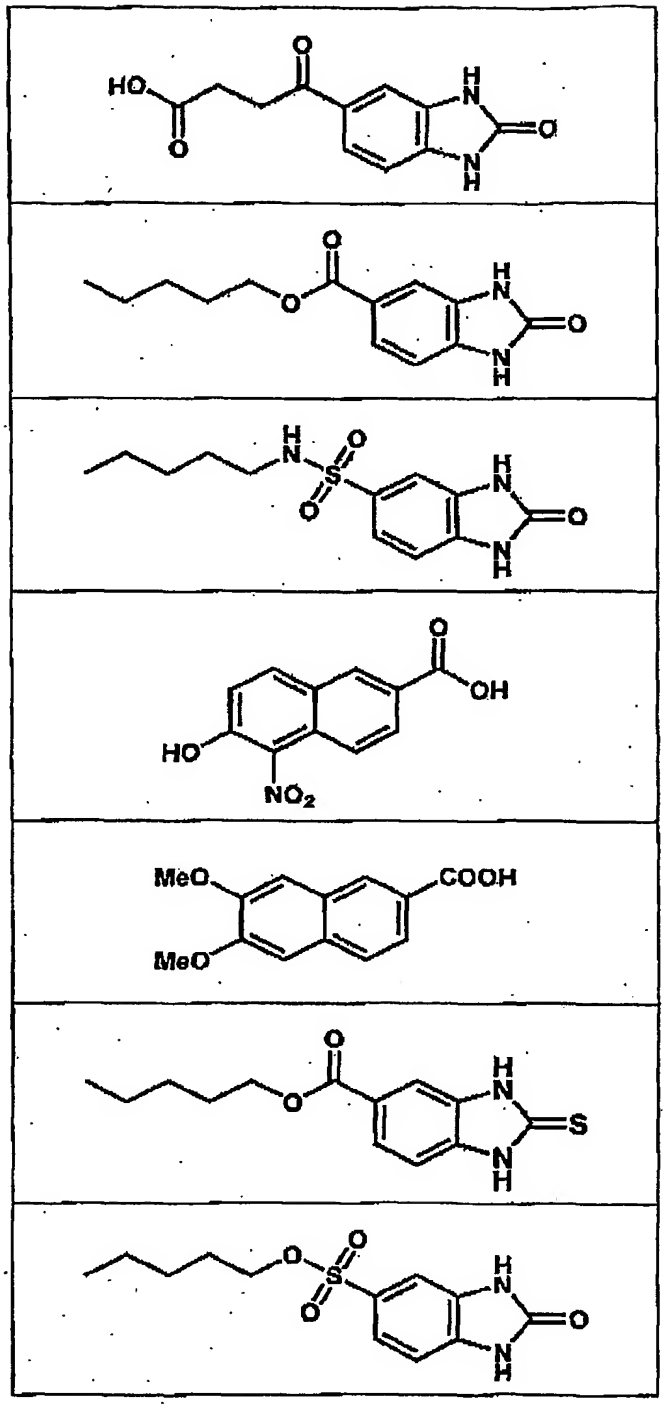
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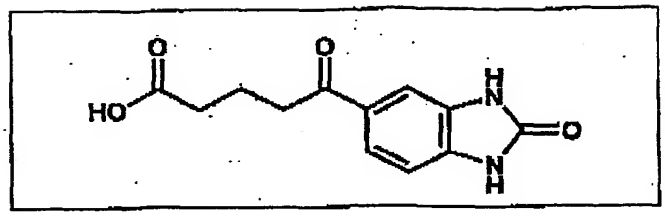


Figure 1

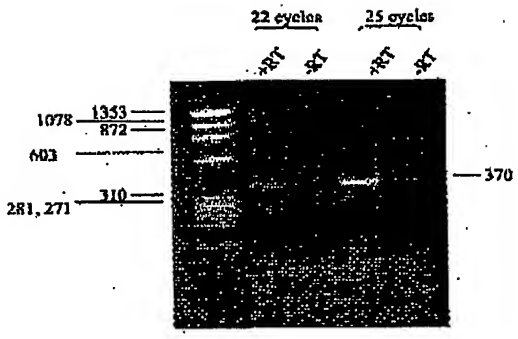
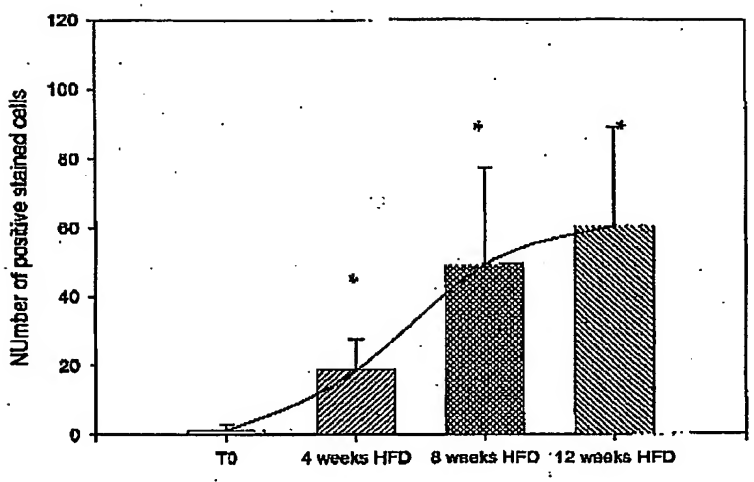

22 cycles
25 cycles
+RT
-RT
+RT
-RT
1078
1353
872
603
310
281, 271
370

Figure 2.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100
80
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40
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NUmber of postive stained cells
*
*
*
T0
4 weeks HFD
8 weeks HFD
12 weeks HFD

Figure 3

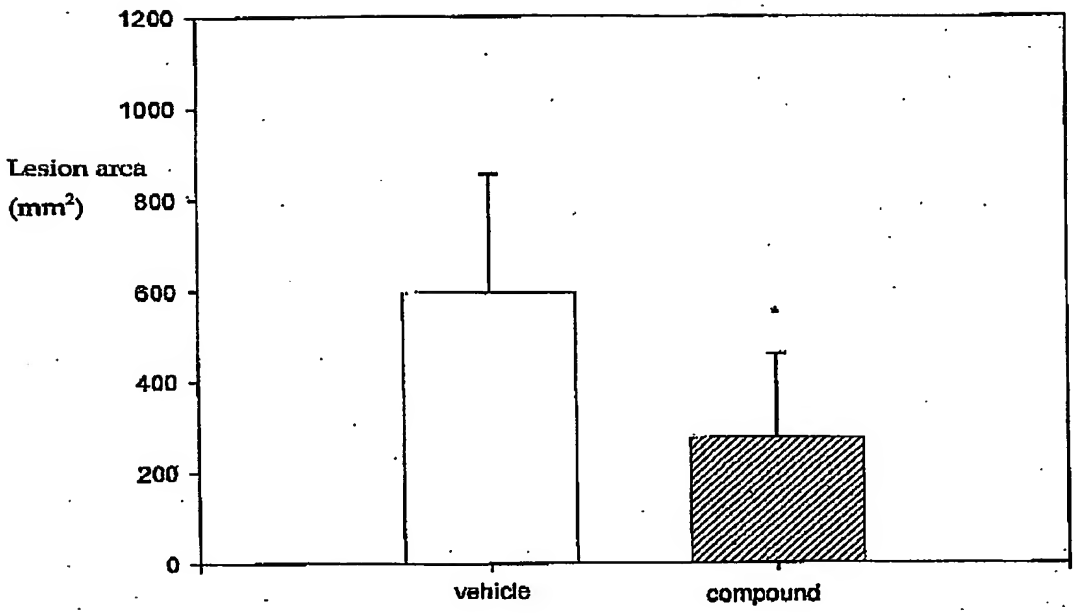


Figure 4

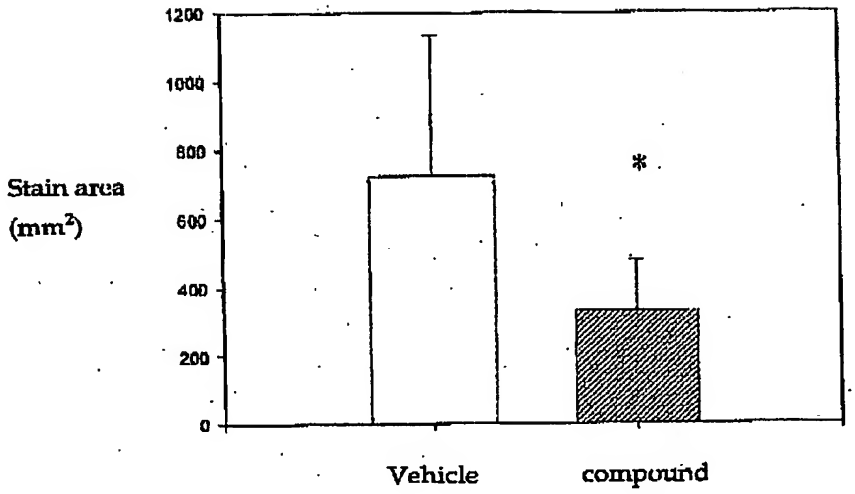


Figure 5

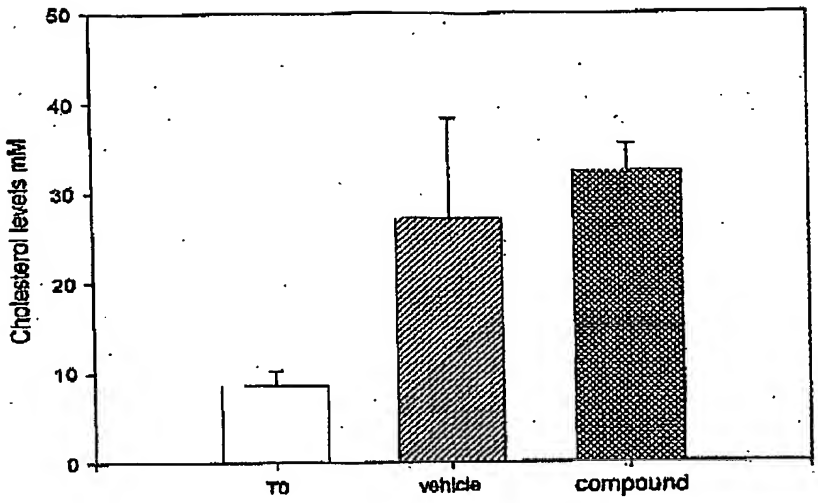


Figure 6

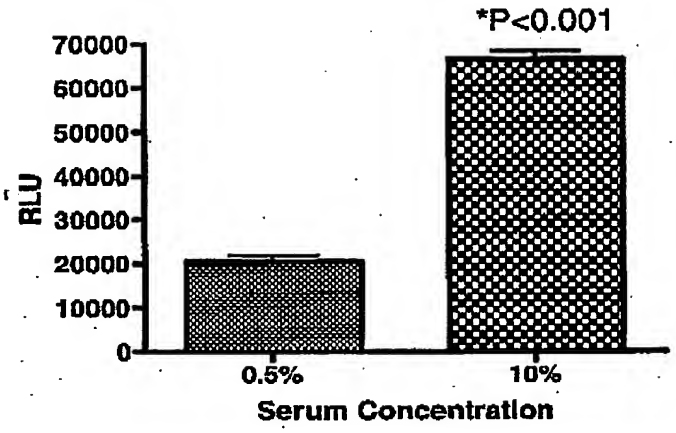

*P<0.001
70000
60000
50000
40000
30000
20000
10000
0
RLU
0.5%
10%
Serum Concentration

Figure 7

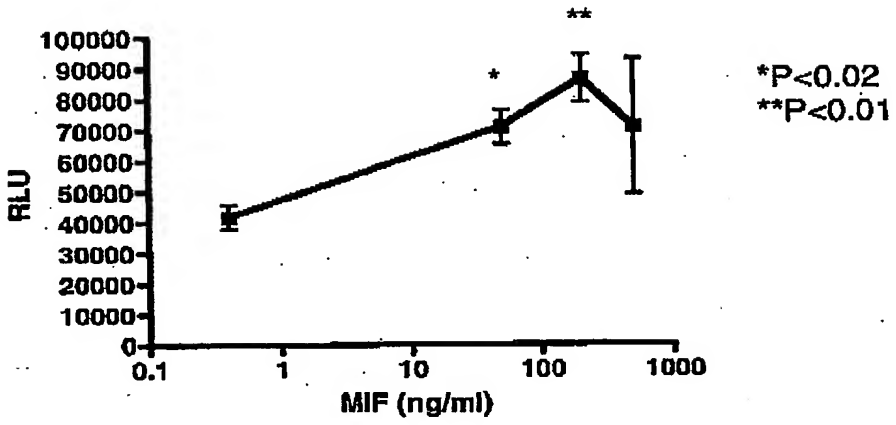

RLU
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0.1
1
10
100
1000
MIF (ng/ml)
*
**
*P<0.02
**P<0.01

Figure 8

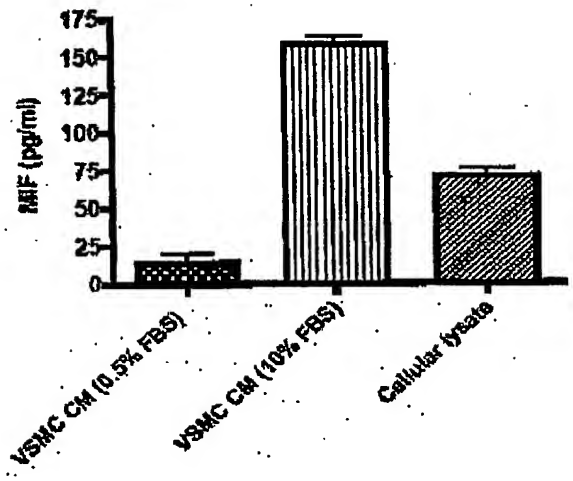

MIF (pg/ml)
175
150
125
100
75
50
25
0
VSMC CM (0.5% FBS)
VSMC CM (10% FBS)
Cellular lysate

Figure 9

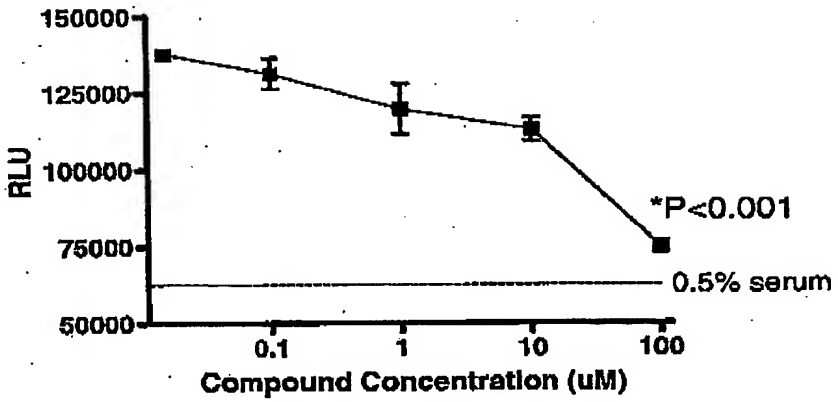

150000
125000
100000
75000
50000
RLU
*P<0.001
0.5% serum
0.1
1
10
100
Compound Concentration (uM)

Figure 10

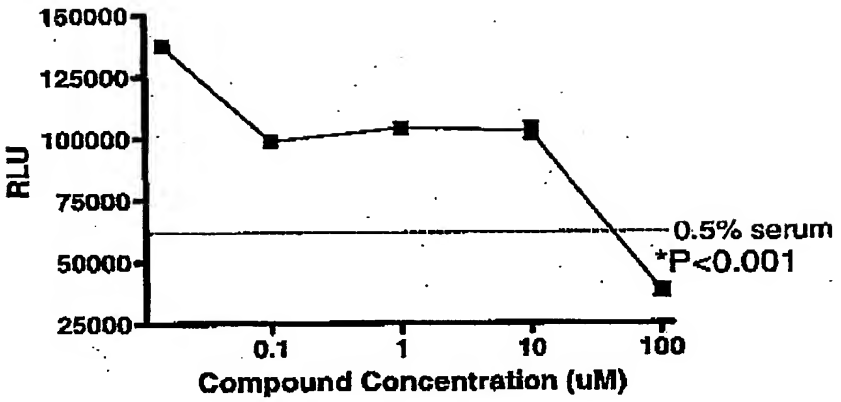

150000
125000
100000
75000
50000
25000
RLU
0.1
1
10
100
Compound Concentration (uM)
0.5% serum
*P<0.001

Figure 11

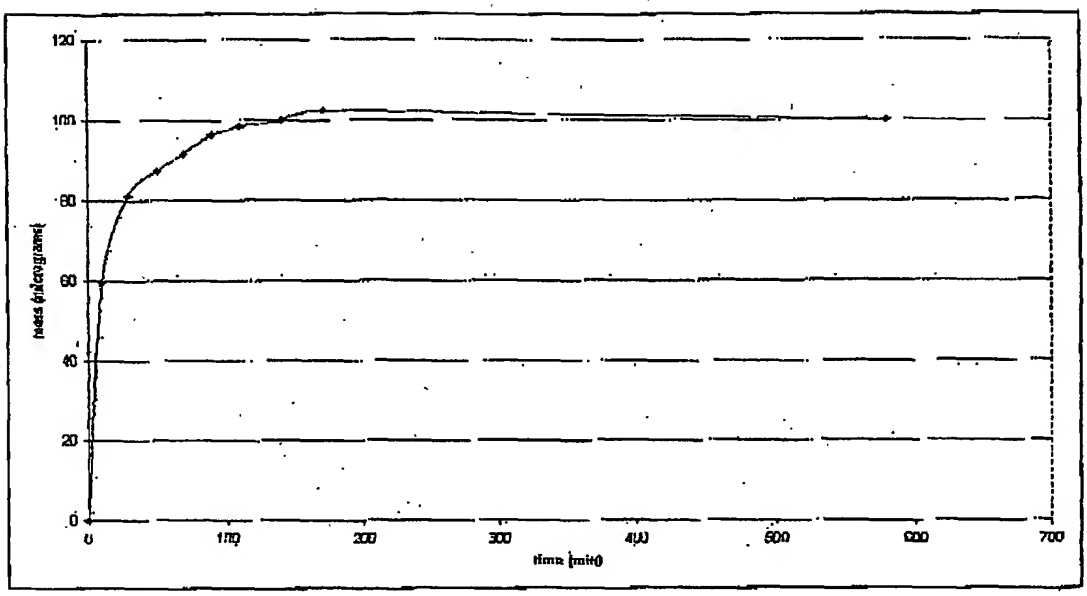
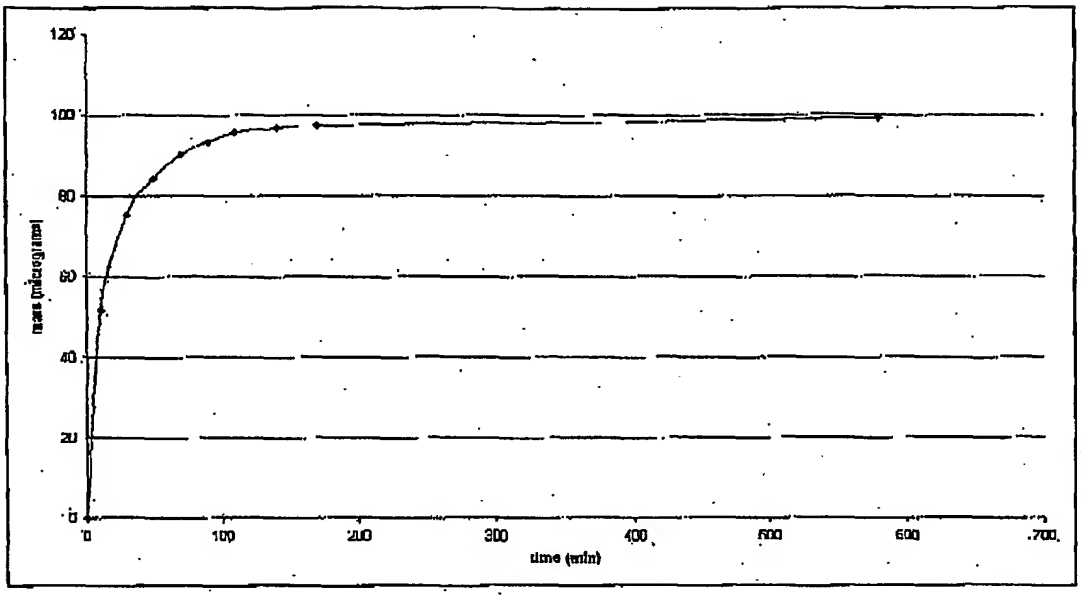


Figure 12


120
100
80
60
40
20
0
mass (micrograms)
0
100
200
300
400
500
600
700
time (min)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001778

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61 K 31/192, 31/4184; A61F 2/06; A61P 9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, USPTO, PubMed: MIF, MMIF, DF, macrophage migration inhibitory factor, antagonist, inhibitor, implant, stent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2004/089927 A1 (Cortical Pty Ltd) 21 October 2004. The whole document, particularly page 37 line 16 – page 38 line 6, page 50 line 3 – page 51 line 27.	1-10
Y	WO 2003/104203 A1 (Cortical Pty Ltd) 18 December 2003. The whole document, particularly page 8 line 9 – page 9 line 16, and claim 21.	1-12
Y	WO 2003/104178 A1 (Cortical Pty Ltd) 18 December 2003. The whole document, particularly page 8 line 8 – page 9 line 15, and claim 21.	1-12
Y	Indolfi C, Mongiardo A, Curcio A, Torella D. Molecular mechanisms of in-stent restenosis and approach to therapy with eluting stents. Trends Cardiovasc Med. 2003 May;13(4):142-8. See the whole document, particularly 2nd full paragraph column 3 page 144 – end of 1st paragraph column 3 page 146.	1-12

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2005	- 1 MAR 2005
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **JASON MACKENZIE** Telephone No : (02) 6283 7934

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001778

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6420188 B1 (Bucala et al.) 16 July 2002. The whole document, particularly column 10 lines 56-59, and column 12 lines 34-43.	1-6
X	US 6492428 B1 (Al-Abed et al.) 10 December 2002. The whole document, particularly column 15 lines 9-15, and column 16 lines 58-67.	1-6
X	US 6599938 B1 (Al-Abed et al.) 29 July 2003. The whole document, particularly column 13 line 61 – column 14 line 21,	1-6
X	WO 1994/026307 A1 (The Picower Institute for Medical Research) 24 November 1994. The whole document, particularly pages 26-53.	1-6
X	WO 1998/017314 A1 (The Picower Institute for Medical Research) 30 April 1998. The whole document, particularly the 3rd full paragraph on page 10, and the 4th full paragraph on page 12.	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2004/001778

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2004089927						
WO	03104203	WO	03104178				
US	6420188	AU	12356/01	AU	79011/01	CA	2218364
		CA	2389229	CA	2416750	CA	2450589
		EP	0821551	EP	1228037	EP	1311255
		EP	1411930	US	6492428	US	6599938
		US	2003008908	US	2003105014	US	2004204464
		WO	0132606	WO	0207720	WO	9729635
		WO	02100332				
WO	9426307	AU	51014/98	AU	68345/94	CA	2163211
		CA	2267069	EP	0702566	EP	0954334
		US	6030615	US	6080407	US	6645493
		US	6774227	US	2003099653	US	2004053843
		US	2004156848	WO	9817314		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

(11) Application No. AU 2005253142 A1

(19) AUSTRALIAN PATENT OFFICE

(54) Title
Method for the early detection of renal disease and injury

(51)[6] International Patent Classification(s)
G01N 33/53 (2006.01) 33/542
G01N 33/542 (2006.01) 20060101ALI2006050 6BMEP G01N
G01N 33/68 (2006.01) 33/68
G01N 33/53 20060101ALI2006072 20060101AFI2006050 2BMEP 6BMEP G01N
PCT/US2005/019951

(21) Application No: 2005253142 (22) Application Date: 2005.06.07

(87) WIPO No: WO05/121788

(30) Priority Data

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11/096,113	2005.03.31	US
60/577,662	2004.06.07	US

(43) Publication Date : 2005.12.22

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